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(54) **Platelet activating factor acetylhydrolase, and gene thereof**

(57) A protein having activities of a human platelet activating factor acetylhydrolase, and represented by an amino acid sequence represented by the following formula (I) or an amino acid sequence having homology therewith; and a DNA encoding the protein:

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Met Gly Val Asn Gln Ser Val Gly Phe Pro Pro Val Thr Gly Pro  
 His Leu Val Gly Cys Gly Asp Val Met Glu Gly Gln Asn Leu Gln  
 Gly Ser Phe Phe Arg Leu Phe Tyr Pro Cys Gln Lys Ala Glu Glu  
 Thr Met Glu Gln Pro Leu Trp Ile Pro Arg Tyr Glu Tyr Cys Thr  
 Gly Leu Ala Glu Tyr Leu Gln Phe Asn Lys Arg Cys Gly Gly Leu  
 Leu Phe Asn Leu Ala Val Gly Ser Cys Arg Leu Pro Val Ser Trp  
 Asn Gly Pro Phe Lys Thr Lys Asp Ser Gly Tyr Pro Leu Ile Ile  
 Phe Ser His Gly Leu Gly Ala Phe Arg Thr Leu Tyr Ser Ala Phe  
 Cys Met Glu Leu Ala Ser Arg Gly Phe Val Val Ala Val Pro Glu  
 His Arg Asp Arg Ser Ala Ala Thr Thr Tyr Phe Cys Lys Gln Ala  
 Pro Glu Glu Asn Gln Pro Thr Asn Glu Ser Leu Gln Glu Glu Trp  
 Ile Pro Phe Arg Arg Val Glu Glu Gly Glu Lys Glu Phe His Val  
 Arg Asn Pro Gln Val His Gln Arg Val Ser Glu Cys Leu Arg Val  
 Leu Lys Ile Leu Gln Glu Val Thr Ala Gly Gln Thr Val Phe Asn  
 Ile Leu Pro Gly Gly Leu Asp Leu Met Thr Leu Lys Gly Asn Ile  
 Asp Met Ser Arg Val Ala Val Met Gly His Ser Phe Gly Gly Ala  
 Thr Ala Ile Leu Ala Leu Ala Lys Glu Thr Gln Phe Arg Cys Ala  
 Val Ala Leu Asp Ala Trp Met Phe Pro Leu Glu Arg Asp Phe Tyr  
 Pro Lys Ala Arg Gly Pro Val Phe Phe Ile Asn Thr Glu Lys Phe

Gln Thr Met Glu Ser Val Asn Leu Met Lys Lys Ile Cys Ala Gln  
 His Glu Gln Ser Arg Ile Ile Thr Val Leu Gly Ser Val His Arg  
 Ser Gln Thr Asp Phe Ala Phe Val Thr Gly Asn Leu Ile Gly Lys  
 Phe Phe Ser Thr Glu Thr Arg Gly Ser Leu Asp Pro Tyr Glu Gly  
 Gln Glu Val Met Val Arg Ala Met Leu Ala Phe Leu Gln Lys His  
 Leu Asp Leu Lys Glu Asp Tyr Asn Gln Trp Asn Asn Leu Ile Glu  
 Gly Ile Gly Pro Ser Leu Thr Pro Gly Ala Pro His His Leu Ser  
 Ser Leu

(1)

**Description**BACKGROUND OF THE INVENTION

## a) Field of the Invention

This invention relates to a novel platelet activating factor acetylhydrolase, and a gene encoding the same.

## b) Description of the Related Art

A platelet activating factor acetylhydrolase is an enzyme, which acts on a platelet activating factor (hereinafter abbreviated as "PAF") and eliminates its 2-acetyl group to deprive PAF of its activity. Since PAF is a mediator for inflammation which causes defluxion of tissue fluid through finer vessels, vasodilation, smooth muscle contraction, endothelial adhesion, activation of neutrophils, macrophages or eosinophilic leukocytes, or the like, PAF acetylhydrolase is usable as a preventive or therapeutic for various diseases caused by PAF.

Some reports have been made about PAF acetylhydrolase to date. For its use as a medicine, however, there is an outstanding desire for the provision of a PAF acetylhydrolase having higher purity and stronger action compared with conventional PAF acetylhydrolase. Further, from the viewpoint of safety, PAF acetylhydrolase derived from human being instead of an animal is desired.

SUMMARY OF THE INVENTION

With the foregoing in view, the present invention has as a primary object the provision of PAF acetylhydrolase which can fulfill the above-described desires.

Interested in the wide-spread distribution of PAF acetylhydrolase in animal organs such as the brain and kidneys, the present inventors chose the bovine liver as a source, and by various isolation and purification procedures, progressively increased the purity of PAF acetylhydrolase while placing a focus on its enzymatic activity. As a result, the present inventors have succeeded in obtaining bovine PAF acetylhydrolase as a pure product and further in determining its amino acid sequence. In addition, from the amino acid sequence of the PAF acetylhydrolase, a gene encoding the enzyme has been found by methods known *per se* in the art.

Moreover, using the bovine PAF acetylhydrolase cDNA, the present inventors have also succeeded in identifying the human PAF acetylhydrolase cDNA.

The present invention has been completed based on these findings, and provides a human PAF acetylhydrolase, which plays an important role as a PAF-inhibiting substance, and also a gene which encodes the enzyme and is important for the synthesis of the enzyme by genetic engineering.

The human PAF acetylhydrolase according to the present invention selectively degrades PAF and hence, is usable as medicines or biochemical reagents for the prevention and treatment of diseases caused by PAF, for example, diseases such as asthma, exudative tympanitis, hemorrhagic colitis and adult respiratory distress syndrome.

DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENTS

The human PAF acetylhydrolase according to the present invention can be prepared as will be described next. PAF acetylhydrolase is first collected from an animal. From the PAF acetylhydrolase, the animal PAF acetylhydrolase cDNA is determined. Using the animal PAF acetylhydrolase cDNA, the human PAF acetylhydrolase cDNA is detected from a human gene library. The human PAF acetylhydrolase cDNA is inserted in an appropriate vector and then cultured in an adequate host organism, whereby the human PAF acetylhydrolase is obtained.

Upon practice of the present invention, it is first necessary to obtain animal PAF acetylhydrolase from an organ of an animal such as the brain, liver or kidneys by purifying it through repetitions of known isolation and purification procedures while using PAF acetylhydrolase activity as an index. A description will hereinafter be made of a process for obtaining PAF acetylhydrolase by using a bovine liver as an example.

As the bovine liver to be used as a source, one obtained from a bovine immediately after its slaughter is preferred.

After the bovine liver is first washed with an appropriate buffer (for example, 10 mM Tris-HCl buffer containing 250 mM sucrose and 1 mM EDTA and having a pH of 7.4), it is homogenized with the same buffer. The homogenate is then centrifuged to obtain a soluble fraction.

Making combined use of hydrophobic chromatography, ion exchange chromatography, adsorption chromatography, gel filtration chromatography and the like, the soluble fraction is purified until a single peak is observed by Mono Q FPLC, so that PAF acetylhydrolase can be obtained.

Incidentally, PAF acetylhydrolase activity which is used as an index for the selective collection of the PAF-acetyl-

hydrolase-containing fraction can be determined, for example, by the method disclosed in Japanese Patent Application Laid-Open (Kokai) No. HEI 7-39373.

With respect to the bovine PAF acetylhydrolase obtained in the above-described manner, its amino acid sequence was investigated by a method known *per se* in the art. As a result, the amino acid sequence has been found to be represented by the following formula (III):

```

Met Gly Val Asn Gln Ser Val Ser Phe Pro Pro Val Thr Gly Pro
His Leu Val Gly Cys Gly Asp Val Met Glu Gly Gln Ser Leu Gln
Gly Ser Phe Phe Arg Leu Phe Tyr Pro Cys Gln Glu Ala Glu Glu
Thr Ser Glu Gln Pro Leu Trp Ile Pro Arg Tyr Glu Tyr Cys Ala
Gly Leu Ala Glu Tyr Leu Lys Phe Asn Lys Arg Trp Gly Gly Leu
Leu Phe Asn Leu Gly Val Gly Ser Cys Arg Leu Pro Val Ser Trp
Asn Gly Pro Phe Lys Thr Lys Asp Ser Gly Tyr Pro Leu Ile Ile
Phe Ser His Gly Met Gly Ala Phe Arg Thr Val Tyr Ser Ala Phe
Cys Met Glu Leu Ala Ser Arg Gly Phe Val Val Ala Val Pro Glu
His Arg Asp Gly Ser Ala Ala Ala Thr Cys Phe Cys Lys Gln Thr
Pro Glu Glu Asn Gln Pro Asp Asn Glu Ala Leu Lys Glu Glu Trp
Ile Pro His Arg Gln Ile Glu Glu Gly Glu Lys Glu Phe Tyr Val
Arg Asn Tyr Gln Val His Gln Arg Val Ser Glu Cys Val Arg Val
Leu Lys Ile Leu Gln Glu Val Thr Ala Gly Gln Ala Val Leu Asn
Ile Leu Pro Gly Gly Leu Asp Leu Met Thr Leu Lys Gly Gly Ile
Asp Val Ser Arg Val Ala Val Met Gly His Ser Phe Gly Gly Ala
Thr Ala Ile Leu Ala Leu Ala Lys Glu Met Gln Phe Arg Cys Ala

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Val Ala Leu Asp Ala Trp Met Phe Pro Leu Glu His Asp Phe Tyr  
 5 Pro Thr Ala Arg Gly Pro Ile Phe Phe Ile Asn Ala Glu Lys Phe  
 Gln Thr Val Glu Thr Val Asn Leu Met Lys Lys Ile Cys Asp Gln  
 His His Gln Ser Arg Ile Ile Thr Val Leu Gly Ser Val His Arg  
 10 Ser Leu Thr Asp Phe Val Phe Val Ala Gly Asn Trp Ile Ser Lys  
 Phe Phe Ser Ser His Thr Arg Gly Ser Leu Asp Pro Tyr Glu Gly  
 15 Gln Glu Thr Val Val Arg Ala Met Leu Ala Phe Leu Gln Lys His  
 Leu Asp Leu Lys Glu Asp Tyr Asp Gln Trp Asn Asn Phe Ile Glu  
 Gly Ile Gly Pro Ser Leu Thr Pro Gly Ala Pro His His Leu Ser  
 20 Ser Leu

(III)

25 Further, from the peptide sequence of the bovine PAF acetylhydrolase of the formula (III), a gene encoding the enzyme was determined by a method known *per se* in the art. The gene (hereinafter called the "bovine PAF acetylhydrolase cDNA") has been found to be identified by the following formula (IV):

30 GTCGACCCACGCGTCCGAGTTGACCGT  
 CTGGGCTGTTTCTGAGGGTCAACGTGACTCGCCGTCAAGTTCAGCCACTGCCCAAGTCGT  
 CGTTCAGTTCAGTTGGTTATGAG ATG GGG GTC AAC CAG TCT GTG AGC TTC  
 35 CCA CCC GTC ACG GGA CCC CAC CTC GTA GGC TGT GGG GAT GTG ATG  
 GAG GGT CAG AGC CTC CAG GGC AGC TTC TTT CGA CTG TTC TAC CCG  
 40 TGC CAA GAG GCA GAG GAG ACC TCG GAG CAG CCC CTG TGG ATT CCC  
 CGC TAT GAG TAC TGC GCT GGC CTG GCC GAA TAC CTA AAG TTT AAT  
 AAG CGC TGG GGG GGG TTA CTG TTC AAC CTG GGT GTG GGA TCT TGT  
 45 CGC CTG CCT GTT AGC TGG AAT GGC CCC TTT AAA ACA AAG GAC TCT  
 GGA TAC CCC TTG ATC ATC TTC TCT CAT GGC ATG GGA GCC TTC AGG

50

55

5 ACA GTG TAT TCA GCC TTC TGC ATG GAG CTG GCT TCT CGT GGC TTT  
 GTG GTT GCT GTA CCA GAG CAC AGG GAT GGG TCA GCT GCG GCC ACC  
 TGT TTC TGC AAG CAG ACC CCA GAG GAG AAC CAG CCT GAC AAT GAG  
 10 GCC CTG AAG GAG GAA TGG ATC CCC CAC CGT CAA ATT GAG GAA GGG  
 GAG AAG GAA TTC TAT GTT CGG AAC TAC CAG GTG CAT CAG AGG GTG  
 AGC GAG TGT GTG AGG GTG TTG AAG ATC CTA CAA GAG GTC ACT GCT  
 15 GGG CAG GCC GTT CTC AAC ATC TTG CCT GGC GGA TTG GAT CTG ATG  
 ACC TTG AAG GGC GGC ATT GAC GTG AGC CGT GTG GCT GTA ATG GGA  
 CAT TCA TTT GGA GGG GCC ACA GCT ATT CTG GCC TTG GCC AAG GAG  
 20 ATG CAA TTT AGG TGT GCT GTG GCT TTG GAC GCT TGG ATG TTT CCT  
 CTG GAG CAT GAC TTT TAC CCC ACG GCC CGA GGC CCT ATC TTC TTT  
 25 ATC AAT GCT GAG AAG TTC CAG ACA GTG GAG ACT GTC AAC TTG ATG  
 AAA AAG ATT TGT GAC CAG CAC CAC CAA TCC AGG ATC ATA ACT GTC  
 CTT GGT TCT GTT CAT CGG AGT CTA ACC GAC TTT GTT TTT GTG GCT  
 30 GGT AAC TGG ATT AGT AAA TTC TTC TCC AGT CAC ACC CGT GGA AGC  
 TTG GAC CCC TAT GAA GGT CAG GAG ACC GTG GTG CGG GCC ATG TTG  
 35 GCC TTC CTG CAG AAG CAT CTT GAC CTG AAA GAG GAC TAT GAC CAG  
 TGG AAC AAC TTC ATT GAA GGC ATT GGC CCA TCA CTG ACC CCA GGG  
 GCC CCA CAC CAT CTG TCC AGC CTG TAG GCACAACCTGGTCATCTTGTGGAAG  
 40 GTCCCTGAGCTGAGTTCCCGTGTGGGGCCTGCCAGGGATACCCTTGGCCTCCTATCAGG  
 AAGTGATTGCCATGACCCTTCTGTGTTGATTGAGAGGATATAATCACACTGCTGATTGGT  
 AACGGGGTACTTGGATTCTCAGACTTGTGATCTTAACTCATGTTGGGACTTGGGTTCA  
 45 CTTACTGATGGGCAAACGGGCATTCTGAGGACTGAGCCTTAATGGTATGGAGAACAAACA  
 GTGGGATGGGGCTGGGGAAGATCTAAGCCCTAAGCTGGGCACTATGAGCCCTATAAACCC  
 50 AACCAGCCAACACCCTCACCTTGGGCAAGTATGACTTCTGCAGGTCGACTCT

( IV )

55 To obtain human PAF acetylhydrolase from the bovine PAF acetylhydrolase cDNA obtained as described above, the human gene library is screened by a method known *per se* in the art while using the bovine PAF acetylhydrolase cDNA as a template.

Described specifically, the bovine PAF acetylhydrolase cDNA is labeled, for example, by incorporating fluorescein-

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12-dUTP through PCR. By the colony hybridization technique that selects each positive colony by ECL (Enhanced Chemiluminescence; Amersham K.K.), colonies containing the human PAF acetylhydrolase cDNA can be obtained.

The human PAF acetylhydrolase cDNA obtained as described above has been found to be identified by the following formula (II):

5

GCAGGTCTCGACCCACGCGTCCGCGGACGCGTGGG

10

CGAGAAGTGCTTCCAAGCGTCCATTTTGAGCCTTGGAACCTACGACGACCAAAGGGCCAC

GGGTTCTGCGTTCGTTTCTCATTTCCGTCGAGTTAAACGTCTGGGGCTGCTTCTGAGGAA

TCAGCTTGGCTGGCCAGCAAGTTCAGCTCCGGCAAGTCATTTGATTACCCCGGTGATGAA

15

ATG GGG GTC AAC CAG TCT GTG GGC TTT CCA CCT GTC ACA GGA CCC

CAC CTC GTA GGC TGT GGG GAT GTG ATG GAG GGT CAG AAT CTC CAG

GGG AGC TTC TTT CGA CTC TTC TAC CCC TGC CAA AAG GCA GAG GAG

20

ACC ATG GAG CAG CCC CTG TGG ATT CCC CGC TAT GAG TAC TGC ACT

GGC CTG GCC GAG TAC CTG CAG TTT AAT AAG CGC TGC GGG GGC TTG

25

CTG TTC AAC CTG GCG GTG GGA TCT TGT CGC CTG CCT GTT AGC TGG

AAT GGC CCC TTT AAG ACA AAG GAC TCT GGA TAC CCC TTG ATC ATC

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55

5 TTC TCC CAT GGC CTA GGA GCC TTC AGG ACT TTG TAT TCA GCC TTC  
 TGC ATG GAG CTG GCC TCA CGT GGC TTT GTG GTT GCT GTG CCA GAG  
 CAC AGG GAC CGG TCA GCG GCA ACC ACC TAT TTC TGC AAG CAG GCC  
 10 CCA GAA GAG AAC CAG CCC ACC AAT GAA TCG CTG CAG GAG GAA TGG  
 ATC CCT TTC CGT CGA GTT GAG GAA GGG GAG AAG GAA TTT CAT GTT  
 CGG AAT CCC CAG GTG CAT CAG CGG GTA AGC GAG TGT TTA CGG GTG  
 15 TTG AAG ATC CTG CAA GAG GTC ACT GCT GGG CAG ACT GTC TTC AAC  
 ATC TTG CCT GGT GGC TTG GAT CTG ATG ACT TTG AAG GGC AAC ATT  
 GAC ATG AGC CGT GTG GCT GTG ATG GGA CAT TCA TTT GGA GGG GCC  
 20 ACA GCT ATT CTG GCT TTG GCC AAG GAG ACC CAA TTT CGG TGT GCG  
 GTG GCT CTG GAT GCT TGG ATG TTT CCT CTG GAA CGT GAC TTT TAC  
 25 CCC AAG GCC CGA GGA CCT GTG TTC TTT ATC AAT ACT GAG AAA TTC  
 CAG ACA ATG GAG AGT GTC AAT TTG ATG AAG AAG ATA TGT GCC CAG  
 CAT GAA CAG TCT AGG ATC ATA ACC GTT CTT GGT TCT GTT CAT CGG  
 30 AGT CAA ACT GAC TTT GCT TTT GTG ACT GGC AAC TTG ATT GGT AAA  
 TTC TTC TCC ACT GAA ACC CGT GGG AGC CTG GAC CCC TAT GAA GGG  
 35 CAG GAG GTT ATG GTA CGG GCC ATG TTG GCC TTC CTG CAG AAG CAC  
 CTC GAC CTG AAA GAA GAC TAT AAT CAA TGG AAC AAC CTT ATT GAA  
 GGC ATT GGA CCG TCG CTC ACC CCA GGG GCC CCC CAC CAT CTG TCC  
 40 AGC CTG TAG GCACAACCTGGCCATTTGTAAAGTCACTTCAGCCAAGTTTTCATTTGGG  
 AGCTACCCAAGGGCACCCATGAGCTCCTATCAAGAAGTGATCAACGTGACCCCTTTTCAC  
 45 AGATTGAAAGGTGTAATCACACTGCTGCTTGGATAACTGGGTACTTTGATCTTAGATTG  
 ATCTTAAATCACTTTGGGACTGGGATCCCTTGCTGATTGACAAACAGACTTTCTGGGAC  
 CTTGATGGAGTGGGGAACAAGCAGTAGAGTGGGACTGGGGGAGACCCAGGCCCCGGGCTG  
 50 AGCACTGTGAGGCCTGGATGTGAAGACTCAGCCCAGCGAAGCTCATTCCTTACCCCCGG

55

CCAGTGCTGCTGCTTCAGTGGAAGAGATGAAGCCAAAGGACAGAATGAAAAATCCCTACCT  
 TCAGAGACTCTAGCCCAGCCCAACACCATCTCTTCCTACCTCTCAGCCTTCTCCCTCCCC  
 5 AGGGCCACTTGTGAAGTCTGAGCACTTTATGTAAATTTCTAGGTGTGAGCCGTGATCAC  
 ATTTTCTATTTATTTCCAAGTCTTCTCATTGTATGGAACATAGTACTACTTATACTTACA  
 10 GTAGTAAGTTATACTTGTGAGCCACAGAGTGGCAGACAGCATGGCTCTCACAGCACAGG  
 GAGAAAACTGAGGTACACAGAGGTACCTCAGAAGCTCTGGATGTCTTTGGGGGTTTTTGC  
 TAAGTGTATCTTGATAGGAAACAACAAAAGCAGGTTGAGATGGGGAAGATGACAGAACAA  
 15 CAGTGTTAAATGGCCATTTGCACAGGCCTTTGCCACAACAGAGAAGTAGTTTGGTCAGCT  
 AAAACTCAGCTGCAGCCTGGACAGTAGAGCGAGACCCCATCTTAAAAATAAAGAAGGCTG  
 20 GGGCTGGTGGCTCATGCCTGTAATCCCAGCACTTTGGGAGGCCAAGGCAGGCAGATCACT  
 TAAGGCCAGGAGTTCAAGACCACCTGGCCAACATGGTGAAACCCCGTCTCTACTAAAAAT  
 ACAAAAAATTAGCCTGGCGTAATGGCAGGCGCCTATAATCCCAGCTACTCAGGAGGCTGA  
 25 AGCAGAAGAATCACTTGAACCTAGGAGGCGGAGGTTGCAGTGAGTCAAGATCGCGCCACT  
 GCACTCCAGCCTGGGTGACAGAGCAAGACTCTGTCTT

( II )

Following conventional procedures, the human PAF acetylhydrolase cDNA obtained as described above is next introduced in an appropriate vector plasmid, and host cells such as mammal cells are then transformed by a commonly-employed recombinant DNA technique to express the human PAF acetylhydrolase. The expression of the human PAF acetylhydrolase can be confirmed by a western blot technique which makes use of an anti-human PAF acetylhydrolase antibody. The introduction into the plasmid, the establishment of the transformed strain, the culture of the strain and the like can be conducted by the general recombinant DNA technology.

From expression systems known to artisans, a suitable expression system can be selected for use in the present invention. It is possible to improve the efficiency of secretion and the level of expression by adding or improving a signal sequence and/or choosing an appropriate host. Although no particular limitation is imposed on host cells, illustrative examples include cultured cells of bacteria, yeasts, other fungi, human and other animals, and cultured cells of plants. Namely, the polynucleotide according to the present invention is inserted in a suitable expression vector, for example, pUC-PL-cl vector, the expression vector is introduced in adequate host cells, for example, *E. Coli* W3110 or the like, and the host cells are then cultured. The target human PAF acetylhydrolase can thereafter be collected as a protein from the thus-obtained cultured matter (cells or culture medium).

As the host, a procaryote or an eucaryote can be used. Usable examples of the procaryote include bacteria, especially *Escherichia coli* and *Bacillus* bacteria, for example, *B. subtilis*. On the other hand, usable examples of the eucaryote include eucaryotic microorganisms such as yeasts, for example, *Saccharomyces* yeasts, especially *s. Servisiae*; insect cells such as armyworm (*Spodoptera Frugiperda*) cells and silkworm (*Bombyx mori*) cells; and animal cells such as human cells, monkey cells and mouse cells, especially monkey cells, for example, COS1 and COS 7.

Usable examples of the expression vector include plasmids, phages, phagemids, viruses [baculoviruses (for insect cells), vaccinia viruses (for animal cells)]. The promoter in the expression vector is selected depending on the host cells. For examples, lac promoters, trp promoters, trc promoters and the like can be used as promoters for bacteria; and adh 1 promoters, pgk promoters and the like can be used as promoters for yeasts. Further, baculovirus polyhedrin promoters can be mentioned as promoters for insects; and early and late promoters of *Simian virus* 40 (SV40) can be mentioned as promoters for animal cells.

When an enhancer is used, for example, the enhancer of SV40 is inserted either upstream or downstream of the gene.

The transformation of the host by the expression vector can be conducted by a common method known *per se* in the art. Such methods are disclosed, for example, in "Current Protocols in Molecular Biology", John Wiley & Sons, Inc.

The culture of the transformants can also be conducted by a usual method. The purification of the human PAF acetylhydrolase from the cultured matter can be conducted following procedures commonly employed for the isolation and purification of proteins, for example, by ultrafiltration and/or one or more of various column chromatographic procedures, for example, chromatography making use of "Sephacrose".

In the above-described manner, the human PAF acetylhydrolase can be advantageously obtained. The human PAF acetylhydrolase according to the present invention is represented by the following formula (I):

```

Met Gly Val Asn Gln Ser Val Gly Phe Pro Pro Val Thr Gly Pro
His Leu Val Gly Cys Gly Asp Val Met Glu Gly Gln Asn Leu Gln
Gly Ser Phe Phe Arg Leu Phe Tyr Pro Cys Gln Lys Ala Glu Glu
Thr Met Glu Gln Pro Leu Trp Ile Pro Arg Tyr Glu Tyr Cys Thr
Gly Leu Ala Glu Tyr Leu Gln Phe Asn Lys Arg Cys Gly Gly Leu
Leu Phe Asn Leu Ala Val Gly Ser Cys Arg Leu Pro Val Ser Trp
Asn Gly Pro Phe Lys Thr Lys Asp Ser Gly Tyr Pro Leu Ile Ile
Phe Ser His Gly Leu Gly Ala Phe Arg Thr Leu Tyr Ser Ala Phe
Cys Met Glu Leu Ala Ser Arg Gly Phe Val Val Ala Val Pro Glu
His Arg Asp Arg Ser Ala Ala Thr Thr Tyr Phe Cys Lys Gln Ala
Pro Glu Glu Asn Gln Pro Thr Asn Glu Ser Leu Gln Glu Glu Trp
Ile Pro Phe Arg Arg Val Glu Glu Gly Glu Lys Glu Phe His Val
Arg Asn Pro Gln Val His Gln Arg Val Ser Glu Cys Leu Arg Val
Leu Lys Ile Leu Gln Glu Val Thr Ala Gly Gln Thr Val Phe Asn

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Ile Leu Pro Gly Gly Leu Asp Leu Met Thr Leu Lys Gly Asn Ile  
 Asp Met Ser Arg Val Ala Val Met Gly His Ser Phe Gly Gly Ala  
 5 Thr Ala Ile Leu Ala Leu Ala Lys Glu Thr Gln Phe Arg Cys Ala  
 Val Ala Leu Asp Ala Trp Met Phe Pro Leu Glu Arg Asp Phe Tyr  
 10 Pro Lys Ala Arg Gly Pro Val Phe Phe Ile Asn Thr Glu Lys Phe  
 Gln Thr Met Glu Ser Val Asn Leu Met Lys Lys Ile Cys Ala Gln  
 His Glu Gln Ser Arg Ile Ile Thr Val Leu Gly Ser Val His Arg  
 15 Ser Gln Thr Asp Phe Ala Phe Val Thr Gly Asn Leu Ile Gly Lys  
 Phe Phe Ser Thr Glu Thr Arg Gly Ser Leu Asp Pro Tyr Glu Gly  
 20 Gln Glu Val Met Val Arg Ala Met Leu Ala Phe Leu Gln Lys His  
 Leu Asp Leu Lys Glu Asp Tyr Asn Gln Trp Asn Asn Leu Ile Glu  
 Gly Ile Gly Pro Ser Leu Thr Pro Gly Ala Pro His His Leu Ser  
 25 Ser Leu

( I )

30 The human PAF acetylhydrolase selectively degrades PAF and oxidized phospholipids and has physiologically active effects such anti-inflammatory effects.

Needless to say, the human PAF acetylhydrolase according to the present invention is not limited to the peptide of the formula (I) but includes peptides having homology therewith, namely, peptides having the same function as the peptide represented by the formula (I) despite substitution, deletion, addition or the like of amino acids at parts of their sequences.

35 The bovine PAF acetylhydrolase represented by the formula (III) may be contemplated to be available by gene manipulation in a similar manner as the human PAF acetylhydrolase. As a matter of fact, however, the bovine PAF acetylhydrolase cannot be obtained unless eucaryotic host cells are used.

To obtain the bovine PAF acetylhydrolase by gene manipulation, it is therefore necessary to employ as host cells 40 those derived from an eucaryote and to select and use a vector compatible with the host cells.

An antibody against the human PAF acetylhydrolase or bovine PAF acetylhydrolase (which may hereinafter be collectively called the "PAF acetylhydrolase") according to the present invention can also be obtained following usual procedures.

45 Described specifically, the antibody can be obtained by sensitizing an animal such as a rabbit with the PAF acetylhydrolase, separating its serum and, if necessary, purifying an immunoglobulin fraction from the serum. To enhance the sensitizing ability of the enzyme in this case, the enzyme in a form bound on a carrier protein such as bovine serum albumin (BSA) or methyl BSA may be used as an immunogen.

Upon sensitizing an animal, the enzyme can also be used together with Freund's complete adjuvant (FCA) or Freund's incomplete adjuvant (FICA) to increase the production of the antibody. It is desired to conduct the sensitization 50 of the animal twice or more. The frequency of sensitization can be determined while checking the antibody titer of the serum by test sampling of blood. The whole blood of an immune animal may be used by slaughtering it as needed. As an alternative, an immune animal may be subjected to booster sensitization as many times as needed to maintain a constant antibody titer, and blood samples may be collected in small quantities as needed for immediate use. It is also possible to obtain a monoclonal antibody in a usual manner by sensitizing a mouse with the enzyme and then forming 55 hybridomas from spleen cells and myeloma cells of the sensitized mouse.

The present invention will hereinafter be described in further detail by the following examples and reference examples. It is however to be noted that the present invention are by no means limited by or to these examples.

## Referential Example 1

## Measurement of PAF Acetylhydrolase Activity

(1) Using unlabeled lyso PAF (product of Bachem Feinchemikalien AG), 1-O-[1-<sup>14</sup>C]hexadecyl-lyso PAF (product of New England Nuclear Company; hereinafter called the "labeled lyso PAF") was diluted to 4,000 dpm/nmol.

On the other hand, 1-O-hexadecyl-2-[<sup>3</sup>H-acetyl]-sn-glycero-3-phosphocholine (hereinafter called "<sup>3</sup>H-acetyl PAF") was diluted to 3,200 dpm/nmol with the unlabeled lyso PAF.

A standard culture system for the measurement of PAF acetylhydrolase was composed of 50 mM Tris-HCl (pH 7.4), 5 mM EDTA, 5 mM 2-mercaptoethanol (2-ME) and 20 nmol <sup>3</sup>H-acetyl PAF. The total volume of the sample was 0.25 ml.

(2) Measurement of PAF acetylhydrolase activity was conducted by culturing a test sample in the above-described standard culture system at 37°C for 30 minutes, adding 2.5 ml of chloroform/methanol (4:1 V/V) and 0.25 ml of water to terminate the reaction, and then measuring the radioactivity of a small amount (0.6 ml) of each upper layer to determine the amount of the acetate liberated from the <sup>3</sup>H-acetyl PAF.

## Example 1

## Obtainment of Bovine PAF Acetylhydrolase

(1) A fresh bovine liver was purchased from a slaughterhouse and was then treated within 3 hours of the slaughter. Treatments were all conducted at 0 to 4°C. The liver was homogenized in a Waring blender subsequent to the addition of a homogenizing buffer [10 mM Tris-HCl (pH 7.4), 250 mM sucrose, 1 mM EDTA] in an amount 5 times as much as the liver. The resulting homogenate was centrifuged for 30 minutes under 100,000 x g, followed by the removal of a solid portion. The resultant supernatant was centrifuged further for 1 hour under 100,000 x g, whereby a dissolved portion was obtained (supernatant portion).

(2) The supernatant portion obtained through the procedures (1) was adjusted to 1 M with NaCl. Subsequent to stirring for 15 minutes, the solution was loaded on a "BUTYL TOYOPEARL 650 M" column which had been equilibrated beforehand with a buffer composed of 50 mM Tris-HCl (pH 7.4), 1 mM EDTA and 1 M NaCl. After the column was washed with the same buffer, proteins were eluted with a linear gradient of NaCl (1 to 0 M). PAF acetylhydrolase activity was eluted as a single peak in 1 to 0 M NaCl fractions.

(3) Active fractions from the "BUTYL TOYOPEARL" column were loaded on a "Q-Sepharose" column which had been equilibrated with 10 mM Tris-HCl (pH 7.4), 1 mM EDTA and 20% (V/V) glycerol (buffer A). The column was washed with the buffer A. Proteins were eluted with a linear gradient of NaCl (0 to 500 mM) in the buffer A. The activity was observed in a fraction eluted with about 300 mM NaCl.

(4) The active fraction from the "Q-Sepharose" column was concentrated to about 6 ml in an "Amicon ultrafiltration cell" in which "YM-10" membranes were used. The thus-concentrated fraction was loaded on a "Biogel A-1.5 m" gel filtration column which had been equilibrated beforehand with 10 mM Tris-HCl (pH 7.4), 200 mM NaCl, 5 mM 2-ME, 20% (V/V) glycerol and 0.5 % (W/V) "CHAPS" (buffer B). The activity was eluted as a single peak in a fraction corresponding to a molecular weight of about 40 kDa.

(5) The active fraction from the "Biogel-A 1.5 m" column was loaded on a hydroxyapatite column which had been equilibrated beforehand with 10 mM Tris-HCl (pH 7.4), 5 mM 2-ME, 20% (V/V) glycerol and 0.5% (W/V) "CHAPS" (buffer C). Proteins were eluted with a linear gradient which ranged from the buffer C alone to a buffer C containing 150 mM KH<sub>2</sub>PO<sub>4</sub>. The activity was observed in a fraction which was eluted with about 50 mM KH<sub>2</sub>PO<sub>4</sub>.

(6) The active fraction from the hydroxyapatite column was dialyzed against the buffer C, and was then loaded on an "FPLC Mono Q HR 5/5" column which had been equilibrated beforehand with the buffer C. Proteins were eluted by a linear gradient of NaCl (0 to 500 mM) in the buffer C. The activity was observed in a fraction which was eluted with 250 mM NaCl, and a protein in the fraction was obtained as purified bovine PAF acetylhydrolase.

The total proteins, total activities, purification degrees (in terms of times) and the like in the individual purification steps described above are tabulated below:

Step	Total proteins (mg)	Total activity ( $\mu\text{mol}/\text{min}$ )	Activity per weight (nmon/min/mg)	Degree of purification (times)	yield (%)
Cytoplasm	46000	73.5	1.6	1	100
BUTYL TOYOPEAL	680	16.3	24	15	22
Q Sepharose FF	72.4	8.96	124	78	12
Biogel A-1.5 m	6.93	7.38	1060	670	10
Hydroxyapatite	3.45	5.29	1530	960	7.2
Mono Q FPLC	0.3	2.16	7200	4500	2.9

## Example 2

## Determination of Amino Acid Sequence of Bovine PAF Acetylhydrolase

(1) About 0.2 mg of the purified PAF acetylhydrolase obtained in Example 1 was reduced with 1 mg of dithiothreitol at room temperature for 2 hours, followed by the S-alkylation with 0.6% (W/V) 4-vinylpyridine at room temperature for 2 hours.

Using a 4.6 mm x 250 mm "Vydak 304-1251 C<sub>4</sub>" column which had been equilibrated beforehand with 20% (V/V) acetonitrile containing 0.1% (V/V) trifluoroacetic acid, the reaction mixture was subjected to reverse phase high-performance liquid chromatography (HPLC). Proteins were then eluted with a linear gradient of acetonitrile (20 to 85% V/V) which contained 0.1% (V/V) trifluoroacetic acid.

(2) 40 kDa polypeptide, which had been purified by the HPLC, was dialyzed against a lysylendopeptidase digestive buffer [0.5 M Tris-HCl (pH 8.5) and 4 M urea]. Next, 1 µg of a lysylendopeptidase was added to the sample. After the reaction mixture was incubated for 18 hours at 37°C, the reaction mixture was fractionated by reverse phase HPLC through a 4.6 mm x 250 mm "Vydak 304-1251 C<sub>4</sub>" column while using a linear gradient of acetonitrile (5 to 70% V/V) which contained 0.1% (V/V) trifluoroacetic acid.

(3) The amino acid sequence of a peptide fragment obtained by the reverse phase HPLC was determined by an automated sequencer ("Model 477A", trade name; manufactured by Applied Biosystems, Inc.).

The base sequence of the bovine PAF acetylhydrolase, which was determined from the amino acid sequence of the peptide fragment, was as shown above by the formula (III).

Further, from the peptide sequence (III) of the bovine PAF acetylhydrolase, a gene encoding the enzyme was determined by a method known *per se* in the art. The gene was found to be represented by the formula (IV).

## Example 3

## Cloning of Non-active Human PAF Acetylhydrolase cDNA

Using as a template the bovine PAF acetylhydrolase cDNA obtained in Example 2, fluorescein-12-dUTP was incorporated in 500,000 clones of each of a fetal human liver cDNA library (pRc/CMV) and a human brain cDNA library (pCMV SPORTS) by PCR. The clones were then subjected to colony hybridization while detecting the labeling reagent by ECL, whereby cloning was conducted. As a result, a single positive clone was obtained from the human brain library.

A plasmid DNA was prepared and the base sequence was determined. The clone was a full-length clone which contained ATG encoding initiating methionine. Encoding 43 N-terminal amino acids were the same as the corresponding amino acids in the sequence of the bovine PAF acetylhydrolase up to the 40th amino acid, and there was poly A at the 3' end. A more accurate determination of the base sequence was conducted. As a result, the cDNA was found to consist of 2188 bp and to contain an ORF (open reading frame) consisting of 253 amino acids. Compared with the bovine PAF acetylhydrolase cDNA, 140 amino acids had been deleted. The segment of the deleted 140 amino acids contains a "catalytic triad" of serine, histidine and aspartic acid, which exhibits catalytic activity. The cDNA is therefore not believed to have PAD acetylhydrolase activity.

Hence, a primer was synthesized at positions flanking the deleted region, and PCR was conducted using the library DNA as a template. From the human brain cDNA, two bands were obtained, one corresponding to the above-described cDNA with the 140 amino acids deleted, and the other to a cDNA having substantially the same length as the bovine PAF acetylhydrolase cDNA. From the foregoing, the human brain library DNA was expected to contain, in addition to the above-obtained cDNA, a human PAF acetylhydrolase cDNA which is actually equipped with PAF acetylhydrolase activity.

## Example 4

## Cloning of Human PAF Acetylhydrolase cDNA

The human brain cDNA library was diluted to give 2000 clones per well, followed by incubation on 5 96-well plates. Subpools consisting of 10 wells were prepared, and positive pools were determined by PCR (Pool Nos. 10, 20, 28, and 38). With respect to these subpools, PCR was conducted well after well, so that positive pools were confirmed (Pool Nos. 10-5, 20-10, and 38-12).

Concerning these pools, incubation was conducted on plates subsequent to dilution. Using the non-active human PAF acetylhydrolase cDNA as a probe, cloning was attempted by hybridization. Labeling of the DNA was conducted with fluorescein 12-dUTP by PCR, and detection was carried out by ECL. Positive colonies were obtained from Pool

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Nos. 10-5 and 20-10. Plasmid DNAs of these clones were replicated, and their base sequences were then determined. As a result, a human PAF acetylhydrolase cDNA represented by the formula (II) was obtained from the clones of Pool Nos. 10-5.

Based on the resultant cDNA, the amino acid sequence of the human PAF acetylhydrolase was determined. It was found to be represented by the formula (I). Up to 88%, the sequence was the same as that of the bovine PAF acetylhydrolase (346/392 amino acids). On the other hand, it was 42% identical to that of the plasma human PAF acetylhydrolase (162/392 amino acids).

Further, the above cDNA was incorporated in the pUC-PI-cl vector, introduced in *E. coli* W3110 and then subjected to expression. A band, which corresponded to a protein having a molecular weight of 42 kDa, was detected by SDS-PAGE.

The protein was investigated for activity. Human PAF acetylhydrolase activity was confirmed.

## [Sequence Listing]

5 SEQ. ID. No.: 1

SEQ. LENGTH: 392

10 SEQ. TYPE: amino acid

MOLECULE TYPE: peptide

ORIGINAL SOURCE:

15 ORGANISM: bovine (*Bos taurus*)

SEQUENCE DESCRIPTION:

20 Met Gly Val Asn Gln Ser Val Ser Phe Pro Pro Val Thr Gly Pro

1 5 10 15

25 His Leu Val Gly Cys Gly Asp Val Met Glu Gly Gln Ser Leu Gln

20 25 30

Gly Ser Phe Phe Arg Leu Phe Tyr Pro Cys Gln Glu Ala Glu Glu

30 35 40 45

Thr Ser Glu Gln Pro Leu Trp Ile Pro Arg Tyr Glu Tyr Cys Ala

50 55 60

35 Gly Leu Ala Glu Tyr Leu Lys Phe Asn Lys Arg Trp Gly Gly Leu

65 70 75

40 Leu Phe Asn Leu Gly Val Gly Ser Cys Arg Leu Pro Val Ser Trp

80 85 90

Asn Gly Pro Phe Lys Thr Lys Asp Ser Gly Tyr Pro Leu Ile Ile

45 95 100 105

Phe Ser His Gly Met Gly Ala Phe Arg Thr Val Tyr Ser Ala Phe

50 110 115 120

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	Cys Met Glu Leu Ala Ser Arg Gly Phe Val Val Ala Val Pro Glu	
5	125	130 135
	His Arg Asp Gly Ser Ala Ala Ala Thr Cys Phe Cys Lys Gln Thr	
	140	145 150
10		
	Pro Glu Glu Asn Gln Pro Asp Asn Glu Ala Leu Lys Glu Glu Trp	
	155	160 165
15		
	Ile Pro His Arg Gln Ile Glu Glu Gly Glu Lys Glu Phe Tyr Val	
	170	175 180
20		
	Arg Asn Tyr Gln Val His Gln Arg Val Ser Glu Cys Val Arg Val	
	185	190 195
	Leu Lys Ile Leu Gln Glu Val Thr Ala Gly Gln Ala Val Leu Asn	
25	200	205 210
	Ile Leu Pro Gly Gly Leu Asp Leu Met Thr Leu Lys Gly Gly Ile	
	215	220 225
30		
	Asp Val Ser Arg Val Ala Val Met Gly His Ser Phe Gly Gly Ala	
	230	235 240
35		
	Thr Ala Ile Leu Ala Leu Ala Lys Glu Met Gln Phe Arg Cys Ala	
	245	250 255
	Val Ala Leu Asp Ala Trp Met Phe Pro Leu Glu His Asp Phe Tyr	
40	260	265 270
	Pro Thr Ala Arg Gly Pro Ile Phe Phe Ile Asn Ala Glu Lys Phe	
	275	280 285
45		
	Gln Thr Val Glu Thr Val Asn Leu Met Lys Lys Ile Cys Asp Gln	
	290	295 300
50		
	His His Gln Ser Arg Ile Ile Thr Val Leu Gly Ser Val His Arg	
	305	310 315
55		

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	Ser	Leu	Thr	Asp	Phe	Val	Phe	Val	Ala	Gly	Asn	Trp	Ile	Ser	Lys	
5						320				325					330	
	Phe	Phe	Ser	Ser	His	Thr	Arg	Gly	Ser	Leu	Asp	Pro	Tyr	Glu	Gly	
						335				340					345	
10	Gln	Glu	Thr	Val	Val	Arg	Ala	Met	Leu	Ala	Phe	Leu	Gln	Lys	His	
						350				355					360	
	Leu	Asp	Leu	Lys	Glu	Asp	Tyr	Asp	Gln	Trp	Asn	Asn	Phe	Ile	Glu	
15																
						365				370					375	
	Gly	Ile	Gly	Pro	Ser	Leu	Thr	Pro	Gly	Ala	Pro	His	His	Leu	Ser	
20						380				385					390	
	Ser	Leu														
25						392										
30																
35																
40																
45																
50																
55																

SEQ. ID. No.: 2

SEQ. LENGTH: 1665

SEQ. TYPE: nucleic acid

MOLECULE TYPE: cDNA

ORIGINAL SOURCE:

ORGANISM: bovine (*Bos taurus*)

SEQUENCE DESCRIPTION:

GTGACCCACGCGTCCGAGTTGACCGTCTGGGCTGTTTCTGAGGGTCAAC 50

GTGACTCGCCGTCAAGTTCAGCCACTGCCCAAGTCGTCGTTTCAGTTCAGTTGGTTATGAG 110

ATG GGG GTC AAC CAG TCT GTG AGC TTC CCA CCC GTC ACG GGA CCC 155

Met Gly Val Asn Gln Ser Val Ser Phe Pro Pro Val Thr Gly Pro

1 5 10 15

CAC CTC GTA GGC TGT GGG GAT GTG ATG GAG GGT CAG AGC CTC CAG 200

His Leu Val Gly Cys Gly Asp Val Met Glu Gly Gln Ser Leu Gln

20 25 30

GGC AGC TTC TTT CGA CTG TTC TAC CCG TGC CAA GAG GCA GAG GAG 245

Gly Ser Phe Phe Arg Leu Phe Tyr Pro Cys Gln Glu Ala Glu Glu

35 40 45

ACC TCG GAG CAG CCC CTG TGG ATT CCC CGC TAT GAG TAC TGC GCT 290

Thr Ser Glu Gln Pro Leu Trp Ile Pro Arg Tyr Glu Tyr Cys Ala

50 55 60

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	GGC CTG GCC GAA TAC CTA AAG TTT AAT AAG CGC TGG GGG GGG TTA	335
	Gly Leu Ala Glu Tyr Leu Lys Phe Asn Lys Arg Trp Gly Gly Leu	
5	65 70 75	
10	CTG TTC AAC CTG GGT GTG GGA TCT TGT CGC CTG CCT GTT AGC TGG	380
	Leu Phe Asn Leu Gly Val Gly Ser Cys Arg Leu Pro Val Ser Trp	
	80 85 90	
15	AAT GGC CCC TTT AAA ACA AAG GAC TCT GGA TAC CCC TTG ATC ATC	425
	Asn Gly Pro Phe Lys Thr Lys Asp Ser Gly Tyr Pro Leu Ile Ile	
20	95 100 105	
25	TTC TCT CAT GGC ATG GGA GCC TTC AGG ACA GTG TAT TCA GCC TTC	470
	Phe Ser His Gly Met Gly Ala Phe Arg Thr Val Tyr Ser Ala Phe	
	110 115 120	
30	TGC ATG GAG CTG GCT TCT CGT GGC TTT GTG GTT GCT GTA CCA GAG	515
	Cys Met Glu Leu Ala Ser Arg Gly Phe Val Val Ala Val Pro Glu	
35	125 130 135	
40	CAC AGG GAT GGG TCA GCT GCG GCC ACC TGT TTC TGC AAG CAG ACC	560
	His Arg Asp Gly Ser Ala Ala Ala Thr Cys Phe Cys Lys Gln Thr	
	140 145 150	
45	CCA GAG GAG AAC CAG CCT GAC AAT GAG GCC CTG AAG GAG GAA TGG	605
	Pro Glu Glu Asn Gln Pro Asp Asn Glu Ala Leu Lys Glu Glu Trp	
50	155 160 165	
55		

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	ATC CCC CAC CGT CAA ATT GAG GAA GGG GAG AAG GAA TTC TAT GTT	650
5	Ile Pro His Arg Gln Ile Glu Glu Gly Glu Lys Glu Phe Tyr Val	
	170 175 180	
10	CGG AAC TAC CAG GTG CAT CAG AGG GTG AGC GAG TGT GTG AGG GTG	695
	Arg Asn Tyr Gln Val His Gln Arg Val Ser-Glu Cys Val Arg Val	
15	185 190 195	
20	TTG AAG ATC CTA CAA GAG GTC ACT GCT GGG CAG GCC GTT CTC AAC	740
	Leu Lys Ile Leu Gln Glu Val Thr Ala Gly Gln Ala Val Leu Asn	
	200 205 210	
25	ATC TTG CCT GGC GGA TTG GAT CTG ATG ACC TTG AAG GGC GGC ATT	785
	Ile Leu Pro Gly Gly Leu Asp Leu Met Thr Leu Lys Gly Gly Ile	
30	215 220 225	
35	GAC GTG AGC CGT GTG GCT GTA ATG GGA CAT TCA TTT GGA GGG GCC	830
	Asp Val Ser Arg Val Ala Val Met Gly His Ser Phe Gly Gly Ala	
	230 235 240	
40	ACA GCT ATT CTG GCC TTG GCC AAG GAG ATG CAA TTT AGG TGT GCT	875
	Thr Ala Ile Leu Ala Leu Ala Lys Glu Met Gln Phe Arg Cys Ala	
45	245 250 255	
50	GTG GCT TTG GAC GCT TGG ATG TTT CCT CTG GAG CAT GAC TTT TAC	920
	Val Ala Leu Asp Ala Trp Met Phe Pro Leu Glu His Asp Phe Tyr	
	260 265 270	
55		

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	CCC ACG GCC CGA GGC CCT ATC TTC TTT ATC AAT GCT GAG AAG TTC	965
5	Pro Thr Ala Arg Gly Pro Ile Phe Phe Ile Asn Ala Glu Lys Phe	
	275 280 285	
10	CAG ACA GTG GAG ACT GTC AAC TTG ATG AAA AAG ATT TGT GAC CAG	1010
	Gln Thr Val Glu Thr Val Asn Leu Met Lys Lys Ile Cys Asp Gln	
15	290 295 300	
20	CAC CAC CAA TCC AGG ATC ATA ACT GTC CTT GGT TCT GTT CAT CGG	1055
	His His Gln Ser Arg Ile Ile Thr Val Leu Gly Ser Val His Arg	
	305 310 315	
25	AGT CTA ACC GAC TTT GTT TTT GTG GCT GGT AAC TGG ATT AGT AAA	1100
	Ser Leu Thr Asp Phe Val Phe Val Ala Gly Asn Trp Ile Ser Lys	
30	320 325 330	
35	TTC TTC TCC AGT CAC ACC CGT GGA AGC TTG GAC CCC TAT GAA GGT	1145
	Phe Phe Ser Ser His Thr Arg Gly Ser Leu Asp Pro Tyr Glu Gly	
	335 340 345	
40	CAG GAG ACC GTG GTG CGG GCC ATG TTG GCC TTC CTG CAG AAG CAT	1190
	Gln Glu Thr Val Val Arg Ala Met Leu Ala Phe Leu Gln Lys His	
45	350 355 360	
50	CTT GAC CTG AAA GAG GAC TAT GAC CAG TGG AAC AAC TTC ATT GAA	1235
	Leu Asp Leu Lys Glu Asp Tyr Asp Gln Trp Asn Asn Phe Ile Glu	
	365 370 375	
55		

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5 GGC ATT GGC CCA TCA CTG ACC CCA GGG GCC CCA CAC CAT CTG TCC 1280  
 Gly Ile Gly Pro Ser Leu Thr Pro Gly Ala Pro His His Leu Ser  
 380 385 390  
 10 AGC CTG TAG GCACAACTGGTCATCTTGTGGAAGGTCCCTGAGCTGAGTTCCCGTGT 1336  
 Ser Leu  
 15 392  
 20 GGGGCCTGCCCAGGGATACCCTTGGCCTCCTATCAGGAAGTGATTGCCATGACCCTTCTG 1396  
 TGTGATTGAGAGGATATAATCACACTGCTGATTGGTAACGGGGTACTTGGATTCTCAGA 1456  
 25 CTTGTCGATCTTAAACTCATGTTGGGACTTGGGGTTCACCTTACTGATGGGCAAACGGGCAT 1516  
 30 TCTGAGGACTGAGCCTTAATGGTATGGAGAACAAACAGTGGGATGGGGCTGGGGAAGATC 1576  
 35 TAAGCCCTAAGCTGGGCACTATGAGCCCTATAAACCCAACCAGCCAACACCCTCACCTTG 1636  
 40 GGCAAGTATGACTTCTGCAGGTCGACTCT 1665  
 45  
 50  
 55

SEQ. ID. No.: 3

SEQ. LENGTH: 392

SEQ. TYPE: amino acid

MOLECULE TYPE: peptide

ORIGINAL SOURCE:

ORGANISM: human

SEQUENCE DESCRIPTION:

Met Gly Val Asn Gln Ser Val Gly Phe Pro Pro Val Thr Gly Pro

1 5 10 15

His Leu Val Gly Cys Gly Asp Val Met Glu Gly Gln Asn Leu Gln

20 25 30

Gly Ser Phe Phe Arg Leu Phe Tyr Pro Cys Gln Lys Ala Glu Glu

35 40 45

Thr Met Glu Gln Pro Leu Trp Ile Pro Arg Tyr Glu Tyr Cys Thr

50 55 60

Gly Leu Ala Glu Tyr Leu Gln Phe Asn Lys Arg Cys Gly Gly Leu

65 70 75

Leu Phe Asn Leu Ala Val Gly Ser Cys Arg Leu Pro Val Ser Trp

80 85 90

Asn Gly Pro Phe Lys Thr Lys Asp Ser Gly Tyr Pro Leu Ile Ile

95 100 105

Phe Ser His Gly Leu Gly Ala Phe Arg Thr Leu Tyr Ser Ala Phe

110 115 120

Cys Met Glu Leu Ala Ser Arg Gly Phe Val Val Ala Val Pro Glu

125 130 135

His Arg Asp Arg Ser Ala Ala Thr Thr Tyr Phe Cys Lys Gln Ala

140 145 150

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	Pro Glu Glu Asn Gln Pro Thr Asn Glu Ser Leu Gln Glu Glu Trp	
5	155	160 165
	Ile Pro Phe Arg Arg Val Glu Glu Gly Glu Lys Glu Phe His Val	
	170	175 180
10	Arg Asn Pro Gln Val His Gln Arg Val Ser Glu Cys Leu Arg Val	
	185	190 195
	Leu Lys Ile Leu Gln Glu Val Thr Ala Gly Gln Thr Val Phe Asn	
15	200	205 210
	Ile Leu Pro Gly Gly Leu Asp Leu Met Thr Leu Lys Gly Asn Ile	
20	215	220 225
	Asp Met Ser Arg Val Ala Val Met Gly His Ser Phe Gly Gly Ala	
25	230	235 240
	Thr Ala Ile Leu Ala Leu Ala Lys Glu Thr Gln Phe Arg Cys Ala	
	245	250 255
30	Val Ala Leu Asp Ala Trp Met Phe Pro Leu Glu Arg Asp Phe Tyr	
	260	265 270
	Pro Lys Ala Arg Gly Pro Val Phe Phe Ile Asn Thr Glu Lys Phe	
35	275	280 285
	Gln Thr Met Glu Ser Val Asn Leu Met Lys Lys Ile Cys Ala Gln	
40	290	295 300
	His Glu Gln Ser Arg Ile Ile Thr Val Leu Gly Ser Val His Arg	
	305	310 315
45	Ser Gln Thr Asp Phe Ala Phe Val Thr Gly Asn Leu Ile Gly Lys	
	320	325 330
50	Phe Phe Ser Thr Glu Thr Arg Gly Ser Leu Asp Pro Tyr Glu Gly	
	335	340 345

55

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[illegible]

SEQ. ID. No.: 4

SEQ. LENGTH: 2559

SEQ. TYPE: nucleic acid

MOLECULE TYPE: cDNA

ORIGINAL SOURCE:

ORGANISM: human

SEQUENCE DESCRIPTION:

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                                GCAGGTCTCGACCCACGCGTCCGCGGACGCGTGGG    35
                                CGAGAAGTGCTTCCAAGCGTCCATTTTGAGCCTTGGAAGTACGACGACCAAAGGGCCAC    95
                                GGGTTCCTGGGTGCTTTCTCATTTCCGTCGAGTTAAACGTCTGGGGCTGCTTCTGAGGAA    155
                                TCAGCTTGGCTGGCCAGCAAGTTCAGCTCCGGCAAGTCATTTGATTACCCGGTGATGAA    215
                                ATG GGG GTC AAC CAG TCT GTG GGC TTT CCA CCT GTC ACA GGA CCC    260
                                Met Gly Val Asn Gln Ser Val Gly Phe Pro Pro Val Thr Gly Pro
                                1           5           10           15
                                CAC CTC GTA GGC TGT GGG GAT GTG ATG GAG GGT CAG AAT CTC CAG    305
                                His Leu Val Gly Cys Gly Asp Val Met Glu Gly Gln Asn Leu Gln
                                20           25           30
                                GGG AGC TTC TTT CGA CTC TTC TAC CCC TGC CAA AAG GCA GAG GAG    350
                                Gly Ser Phe Phe Arg Leu Phe Tyr Pro Cys Gln Lys Ala Glu Glu
                                35           40           45

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	ACC ATG GAG CAG CCC CTG TGG ATT CCC CGC TAT GAG TAC TGC ACT	395
5	Thr Met Glu Gln Pro Leu Trp Ile Pro Arg Tyr Glu Tyr Cys Thr	
	50 55 60	
10	GGC CTG GCC GAG TAC CTG CAG TTT AAT AAG CGC TGC GGG GGC TTG	440
	Gly Leu Ala Glu Tyr Leu Gln Phe Asn Lys Arg Cys Gly Gly Leu	
15	65 70 75	
20	CTG TTC AAC CTG GCG GTG GGA TCT TGT CGC CTG CCT GTT AGC TGG	495
	Leu Phe Asn Leu Ala Val Gly Ser Cys Arg Leu Pro Val Ser Trp	
	80 85 90	
25	AAT GGC CCC TTT AAG ACA AAG GAC TCT GGA TAC CCC TTG ATC ATC	540
	Asn Gly Pro Phe Lys Thr Lys Asp Ser Gly Tyr Pro Leu Ile Ile	
30	95 100 105	
35	TTC TCC CAT GGC CTA GGA GCC TTC AGG ACT TTG TAT TCA GCC TTC	585
	Phe Ser His Gly Leu Gly Ala Phe Arg Thr Leu Tyr Ser Ala Phe	
	110 115 120	
40	TGC ATG GAG CTG GCC TCA CGT GGC TTT GTG GTT GCT GTG CCA GAG	630
	Cys Met Glu Leu Ala Ser Arg Gly Phe Val Val Ala Val Pro Glu	
45	125 130 135	
50	CAC AGG GAC CGG TCA GCG GCA ACC ACC TAT TTC TGC AAG CAG GCC	675
	His Arg Asp Arg Ser Ala Ala Thr Thr Tyr Phe Cys Lys Gln Ala	
	140 145 150	
55		

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	CCA GAA GAG AAC CAG CCC ACC AAT GAA TCG CTG CAG GAG GAA TGG	720
5	Pro Glu Glu Asn Gln Pro Thr Asn Glu Ser Leu Gln Glu Glu Trp	
	155 160 165	
10	ATC CCT TTC CGT CGA GTT GAG GAA GGG GAG AAG GAA TTT CAT GTT	765
	Ile Pro Phe Arg Arg Val Glu Glu Gly Glu Lys Glu Phe His Val	
15	170 175 180	
20	CGG AAT CCC CAG GTG CAT CAG CGG GTA AGC GAG TGT TTA CGG GTG	810
	Arg Asn Pro Gln Val His Gln Arg Val Ser Glu Cys Leu Arg Val	
	185 190 195	
25	TTG AAG ATC CTG CAA GAG GTC ACT GCT GGG CAG ACT GTC TTC AAC	855
	Leu Lys Ile Leu Gln Glu Val Thr Ala Gly Gln Thr Val Phe Asn	
30	200 205 210	
35	ATC TTG CCT GGT GGC TTG GAT CTG ATG ACT TTG AAG GGC AAC ATT	900
	Ile Leu Pro Gly Gly Leu Asp Leu Met Thr Leu Lys Gly Asn Ile	
	215 220 225	
40	GAC ATG AGC CGT GTG GCT GTG ATG GGA CAT TCA TTT GGA GGG GCC	945
	Asp Met Ser Arg Val Ala Val Met Gly His Ser Phe Gly Gly Ala	
45	230 235 240	
50	ACA GCT ATT CTG GCT TTG GCC AAG GAG ACC CAA TTT CGG TGT GCG	990
	Thr Ala Ile Leu Ala Leu Ala Lys Glu Thr Gln Phe Arg Cys Ala	
	245 250 255	
55		

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5 GTG GCT CTG GAT GCT TGG ATG TTT CCT CTG GAA CGT GAC TTT TAC 1035  
Val Ala Leu Asp Ala Trp Met Phe Pro Leu Glu Arg Asp Phe Tyr  
260 265 270

10 CCC AAG GCC CGA GGA CCT GTG TTC TTT ATC AAT ACT GAG AAA TTC 1080  
Pro Lys Ala Arg Gly Pro Val Phe Phe Ile Asn Thr Glu Lys Phe  
15 275 280 285

20 CAG ACA ATG GAG AGT GTC AAT TTG ATG AAG AAG ATA TGT GCC CAG 1125  
Gln Thr Met Glu Ser Val Asn Leu Met Lys Lys Ile Cys Ala Gln  
290 295 300

25 CAT GAA CAG TCT AGG ATC ATA ACC GTT CTT GGT TCT GTT CAT CGG 1170  
His Glu Gln Ser Arg Ile Ile Thr Val Leu Gly Ser Val His Arg  
30 305 310 315

35 AGT CAA ACT GAC TTT GCT TTT GTG ACT GGC AAC TTG ATT GGT AAA 1215  
Ser Gln Thr Asp Phe Ala Phe Val Thr Gly Asn Leu Ile Gly Lys  
320 325 330

40 TTC TTC TCC ACT GAA ACC CGT GGG AGC CTG GAC CCC TAT GAA GGG 1260  
Phe Phe Ser Thr Glu Thr Arg Gly Ser Leu Asp Pro Tyr Glu Gly  
45 335 340 345

50 CAG GAG GTT ATG GTA CGG GCC ATG TTG GCC TTC CTG CAG AAG CAC 1305  
Gln Glu Val Met Val Arg Ala Met Leu Ala Phe Leu Gln Lys His  
350 355 360

55

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5 CTC GAC CTG AAA GAA GAC TAT AAT CAA TGG AAC AAC CTT ATT GAA 1350  
 Leu Asp Leu Lys Glu Asp Tyr Asn Gln Trp Asn Asn Leu Ile Glu  
 365 370 375  
 10 GGC ATT GGA CCG TCG CTC ACC CCA GGG GCC CCC CAC CAT CTG TCC 1395  
 Gly Ile Gly Pro Ser Leu Thr Pro Gly Ala Pro His His Leu Ser  
 15 380 385 390  
 20 AGC CTG TAG GCACAACTGGCCATTTGTAAAGTCACTTCAGCCAAGTTTTTCATTTGGG 1452  
 Ser Leu \*  
 392  
 25 AGCTACCCAAGGGCACCCATGAGCTCCTATCAAGAAGTGATCAACGTGACCCCTTTTCAC 1512  
 30 AGATTGAAAGGTGTAATCACACTGCTGCTTGGATAACTGGGTACTTTGATCTTAGATTTG 1572  
 35 ATCTTAAAATCACTTTGGGACTGGGATCCCTTGCTGATTGACAAACAGACTTTCTGGGAC 1632  
 40 CTTGATGGAGTGGGGAACAAGCAGTAGAGTGGGACTGGGGGAGACCCAGGCCCGGGGCTG 1692  
 45 AGCACTGTGAGGCCTGGATGTGAAGACTCAGCCCAGCGAAGCTCATTCCCTTACCCCCGG 1752  
 50 CCAGTGCTGCTGCTTCAGTGGAAGAGATGAAGCCAAAGGACAGAATGAAAATCCCTACCT 1812  
 55 TCAGAGACTCTAGCCCAGCCCAACACCATCTCTTCCTACCTCTCAGCCTTCTCCCTCCCC 1872

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AGGGCCACTTGTTGAAGTCTGAGCACTTTATGTAAATTTCTAGGTGTGAGCCGTGATCAC 1932

5

ATTTTCTATTTATTTCCAAGTCTTCTCATTGTATGGAACATAGTACTACTTATACTTACA 1992

10

GTAGTAAGTTATACTTGTGAGCCACAGAGTGGCAGACAGCATGGCTCTCACAGCACAGG 2052

15

GAGAAAACTGAGGTACACAGAGGTACCTCAGAAGCTCTGGATGTCTTTGGGGGTTTTGC 2112

20

TAAGTGTATCTTGATAGGAAACAACAAAAGCAGGTTGAGATGGGGAAGATGACAGAACAA 2172

25

AAAACCTCAGCTGCAGCCTGGACAGTAGAGCGAGACCCCATCTTAAAAATAAAGAAGGCTG 2292

30

GGCGTGGTGGCTCATGCCTGTAATCCCAGCACTTTGGGAGGCCAAGGCAGGCAGATCACT 2352

35

TAAGGCCAGGAGTTCAAGACCACCTGGCCAACATGGTGAAACCCGTCTCTACTAAAAAT 2412

40

ACAAAAAATTAGCCTGGCGTAATGGCAGGCGCCTATAATCCCAGCTACTCAGGAGGCTGA 2472

45

AGCAGAAGAATCACTTGAACCTAGGAGGCGGAGGTTGCAGTGAGTCAAGATCGCGCCACT 2532

50

GCACTCCAGCCTGGGTGACAGAGCAAGACTCTGTCTT 2569

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

## (i) APPLICANT:

(A) NAME: SUNTORY LIMITED  
 (B) STREET: 1-40, Dojimahama 2-chome, Kita-ku, Osaka-shi,  
 (C) CITY: OSAKA  
 (E) COUNTRY: JAPAN  
 (F) POSTAL CODE (ZIP): 530

(ii) TITLE OF INVENTION: PLATELET ACTIVATING FACTOR ACETYLHYDROLASE,  
 AND GENE THEREOF

(iii) NUMBER OF SEQUENCES: 4

## (iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
 (B) COMPUTER: IBM PC compatible  
 (C) OPERATING SYSTEM: PC-DOS/MS-DOS  
 (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

## (2) INFORMATION FOR SEQ ID NO: 1:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 392 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (vi) ORIGINAL SOURCE:

(A) ORGANISM: human

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Met Gly Val Asn Gln Ser Val Gly Phe Pro Pro Val Thr Gly Pro His  
 1 5 10 15  
 Leu Val Gly Cys Gly Asp Val Met Glu Gly Gln Asn Leu Gln Gly Ser  
 20 25 30  
 Phe Phe Arg Leu Phe Tyr Pro Cys Gln Lys Ala Glu Glu Thr Met Glu  
 35 40 45  
 Gln Pro Leu Trp Ile Pro Arg Tyr Glu Tyr Cys Thr Gly Leu Ala Glu  
 50 55 60  
 Tyr Leu Gln Phe Asn Lys Arg Cys Gly Gly Leu Leu Phe Asn Leu Ala  
 65 70 75 80  
 Val Gly Ser Cys Arg Leu Pro Val Ser Trp Asn Gly Pro Phe Lys Thr  
 85 90 95  
 Lys Asp Ser Gly Tyr Pro Leu Ile Ile Phe Ser His Gly Leu Gly Ala  
 100 105 110  
 Phe Arg Thr Leu Tyr Ser Ala Phe Cys Met Glu Leu Ala Ser Arg Gly  
 115 120 125

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Phe Val Val Ala Val Pro Glu His Arg Asp Arg Ser Ala Ala Thr Thr  
 130 135 140  
 Tyr Phe Cys Lys Gln Ala Pro Glu Glu Asn Gln Pro Thr Asn Glu Ser  
 145 150 155 160  
 Leu Gln Glu Glu Trp Ile Pro Phe Arg Arg Val Glu Glu Gly Glu Lys  
 165 170 175  
 Glu Phe His Val Arg Asn Pro Gln Val His Gln Arg Val Ser Glu Cys  
 180 185 190  
 Leu Arg Val Leu Lys Ile Leu Gln Glu Val Thr Ala Gly Gln Thr Val  
 195 200 205  
 Phe Asn Ile Leu Pro Gly Gly Leu Asp Leu Met Thr Leu Lys Gly Asn  
 210 215 220  
 Ile Asp Met Ser Arg Val Ala Val Met Gly His Ser Phe Gly Gly Ala  
 225 230 235 240  
 Thr Ala Ile Leu Ala Leu Ala Lys Glu Thr Gln Phe Arg Cys Ala Val  
 245 250 255  
 Ala Leu Asp Ala Trp Met Phe Pro Leu Glu Arg Asp Phe Tyr Pro Lys  
 260 265 270  
 Ala Arg Gly Pro Val Phe Phe Ile Asn Thr Glu Lys Phe Gln Thr Met  
 275 280 285  
 Glu Ser Val Asn Leu Met Lys Lys Ile Cys Ala Gln His Glu Gln Ser  
 290 295 300  
 Arg Ile Ile Thr Val Leu Gly Ser Val His Arg Ser Gln Thr Asp Phe  
 305 310 315 320  
 Ala Phe Val Thr Gly Asn Leu Ile Gly Lys Phe Phe Ser Thr Glu Thr  
 325 330 335  
 Arg Gly Ser Leu Asp Pro Tyr Glu Gly Gln Glu Val Met Val Arg Ala  
 340 345 350  
 Met Leu Ala Phe Leu Gln Lys His Leu Asp Leu Lys Glu Asp Tyr Asn  
 355 360 365  
 Gln Trp Asn Asn Leu Ile Glu Gly Ile Gly Pro Ser Leu Thr Pro Gly  
 370 375 380  
 Ala Pro His His Leu Ser Ser Leu  
 385 390

## (2) INFORMATION FOR SEQ ID NO: 2:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 2559 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:  
 (A) ORGANISM: human

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

	GCAGGTCTCG ACCACGCGT CCGCGGACGC GTGGGCGAGA AGTGCTTCCA AGCGTCCATT	60
5	TTGAGCCTTG GAAACTACGA CGACCAAAGG GCCACGGGTT CCTGGGTCGT TTCTCATTTTC	120
	CGTCGAGTTA AACGTCTGGG GCTGCTTCTG AGGAATCAGC TTGGCTGGCC AGCAAGTTCA	180
	GCTCCGGCAA GTCATTTGAT TCACCCGGTG ATGAAATGGG GGTCAACCAG TCTGTGGGCT	240
10	TTCCACCTGT CACAGGACCC CACCTCGTAG GCTGTGGGGA TGTGATGGAG GGTCAGAATC	300
	TCCAGGGGAG CTTCTTTTGA CTCTTCTACC CCTGCCAAA GGCAGAGGAG ACCATGGAGC	360
	AGCCCCGTGT GATTCCCCGC TATGAGTACT GCACTGGCCT GGCCGAGTAC CTGCAGTTTA	420
15	ATAAGCGCTG CGGGGGCTTG CTGTTCAACC TGGCGGTGGG ATCTTGTCGC CTGCCTGTTA	480
	GCTGGAATGG CCCCTTTAAG ACAAAGGACT CTGGATACCC CTTGATCATC TTCTCCCATG	540
	GCCTAGGAGC CTTCAGGACT TTGTATTCAG CTTTCTGCAT GGAGCTGGCC TCACGTGGCT	600
20	TTGTGGTTGC TGTGCCAGAG CACAGGGACC GGTGAGCGGC AACCACCTAT TTCTGCAAGC	660
	AGGCCCCAGA AGAGAACCAG CCCACCAATG AATCGCTGCA GGAGGAATGG ATCCCTTTTC	720
	GTCGAGTTGA GGAAGGGGAG AAGGAATTTC ATGTTGCGAA TCCCAGGTG CATCAGCGGG	780
25	TAAGCGAGTG TTTACGGGTG TTGAAGATCC TGCAAGAGGT CACTGCTGGG CAGACTGTCT	840
	TCAACATCTT GCCTGGTGGC TTGGATCTGA TGACTTTGAA GGGCAACATT GACATGAGCC	900
	GTGTGGCTGT GATGGGACAT TCATTTGGAG GGGCCACAGC TATTCTGGCT TTGGCCAAGG	960
30	AGACCCAATT TCGGTGTGCG GTGGCTCTGG ATGCTTGGAT GTTTCCTCTG GAACGTGACT	1020
	TTTACCCCAA GGCCCGAGGA CCTGTGTTCT TTATCAATAC TGAGAAATTC CAGACAATGG	1080
	AGAGTGTCOA TTTGATGAAG AAGATATGTG CCCAGCATGA ACAGTCTAGG ATCATAACCG	1140
35	TTCTTGGTTC TGTTCATCGG AGTCAAATG ACTTTGCTTT TGTGACTGGC AACTTGATTG	1200
	GTAAATCTTT CTCCACTGAA ACCCGTGGGA GCCTGGACCC CTATGAAGGG CAGGAGGTTA	1260
	TGGTACGGGC CATGTTGGCC TTCTGCAGA AGCACCTCGA CCTGAAAGAA GACTATAATC	1320
40	AATGGAACAA CCTTATTGAA GGCATTGGAC CGTCGCTCAC CCCAGGGGCC CCCACCATC	1380
	TGTCCAGCCT GTAGGCACAA CTGGCCATTT GTAAAGTCAC TTCAGCCAAG TTTTCATTTG	1440
	GGAGCTACCC AAGGGCACCC ATGAGCTCCT ATCAAGAAGT GATCAACGTG ACCCCTTTTC	1500
45	ACAGATTGAA AGGTGTAATC AACTGCTGC TTGGATAACT GGGTACTTTG ATCTTAGATT	1560
	TGATCTTAAA ATCACTTTGG GACTGGGATC CCTTGCTGAT TGACAAACAG ACTTTCTGGG	1620
	ACCTTGATGG AGTGGGGAAC AAGCAGTAGA GTGGGACTGG GGGAGACCCA GGCCCCGGGC	1680
50	TGAGCACTGT GAGGCCTGGA TGTGAAGACT CAGCCCAGCG AAGCTCATTC CCTTACCCCC	1740
	GGCCAGTGCT GCTGCTTCAG TGGAAGAGAT GAAGCCAAAG GACAGAATGA AAATCCCTAC	1800
	CTTCAGAGAC TCTAGCCCAG CCCAACACCA TCTCTTCCTA CCTCTCAGCC TTCTCCCTCC	1860
55	CCAGGGCCAC TTGTTGAAGT CTGAGCACTT TATGTAAATT TCTAGGTGTG AGCCGTGATC	1920

ACATTTTCTA TTTATTTCCA AGTCTTCTCA TTGTATGGAA CATAGTACTA CTTTACTTTA 1980  
 CAGTAGTAAG TTATACTTGT GAGCCCACAG AGTGGCAGAC AGCATGGCTC TCACAGCACA 2040  
 5 GGGAGAAAAA CTGAGGTACA CAGAGGTACC TCAGAAGCTC TGGATGTCTT TGGGGGTTTT 2100  
 GCTAAGTGTA TCTTGATAGG AAACAACAAA AGCAGGTTGA GATGGGGAAG ATGACAGAAC 2160  
 AACAGTGTTA AATGGCCATT TGCACAGGCC TTTGCCACAA CAGAGAAGTA GTTTGGTCAG 2220  
 10 CTAAACTCA GCTGCAGCCT GGACAGTAGA GCGAGACCCC ATCTTAAAAA TAAAGAAGGC 2280  
 TGGGCGTGGT GGCTCATGCC TGTAAATCCCA GCACCTTGGG AGGCCAAGGC AGGCAGATCA 2340  
 CTTAAGGCCA GGAGTTCAAG ACCACCTGGC CAACATGGTG AAACCCCGTC TCTACTAAAA 2400  
 15 ATACAAAAAA TTAGCCTGGC GTAATGGCAG GCGCCTATAA TCCCAGCTAC TCAGGAGGCT 2460  
 GAAGCAGAAG AATCACTTGA ACCTAGGAGG CGGAGGTTGC AGTGAGTCAA GATCGCGCCA 2520  
 CTGCACTCCA GCCTGGGTGA CAGAGCAAGA CTCTGTCTT 2559

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 392 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: bovine (Bos taurus)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Met Gly Val Asn Gln Ser Val Ser Phe Pro Pro Val Thr Gly Pro His  
 1 5 10 15  
 Leu Val Gly Cys Gly Asp Val Met Glu Gly Gln Ser Leu Gln Gly Ser  
 20 25 30  
 Phe Phe Arg Leu Phe Tyr Pro Cys Gln Glu Ala Glu Glu Thr Ser Glu  
 35 40 45  
 Gln Pro Leu Trp Ile Pro Arg Tyr Glu Tyr Cys Ala Gly Leu Ala Glu  
 50 55 60  
 Tyr Leu Lys Phe Asn Lys Arg Trp Gly Gly Leu Leu Phe Asn Leu Gly  
 65 70 75 80  
 Val Gly Ser Cys Arg Leu Pro Val Ser Trp Asn Gly Pro Phe Lys Thr  
 85 90 95  
 Lys Asp Ser Gly Tyr Pro Leu Ile Ile Phe Ser His Gly Met Gly Ala  
 100 105 110  
 Phe Arg Thr Val Tyr Ser Ala Phe Cys Met Glu Leu Ala Ser Arg Gly  
 115 120 125  
 Phe Val Val Ala Val Pro Glu His Arg Asp Gly Ser Ala Ala Ala Thr  
 130 135 140

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Cys Phe Cys Lys Gln Thr Pro Glu Glu Asn Gln Pro Asp Asn Glu Ala  
 145 150 155 160  
 5 Leu Lys Glu Glu Trp Ile Pro His Arg Gln Ile Glu Glu Gly Glu Lys  
 165 170 175  
 Glu Phe Tyr Val Arg Asn Tyr Gln Val His Gln Arg Val Ser Glu Cys  
 180 185 190  
 10 Val Arg Val Leu Lys Ile Leu Gln Glu Val Thr Ala Gly Gln Ala Val  
 195 200 205  
 Leu Asn Ile Leu Pro Gly Gly Leu Asp Leu Met Thr Leu Lys Gly Gly  
 210 215 220  
 15 Ile Asp Val Ser Arg Val Ala Val Met Gly His Ser Phe Gly Gly Ala  
 225 230 235 240  
 Thr Ala Ile Leu Ala Leu Ala Lys Glu Met Gln Phe Arg Cys Ala Val  
 245 250 255  
 20 Ala Leu Asp Ala Trp Met Phe Pro Leu Glu His Asp Phe Tyr Pro Thr  
 260 265 270  
 Ala Arg Gly Pro Ile Phe Phe Ile Asn Ala Glu Lys Phe Gln Thr Val  
 275 280 285  
 25 Glu Thr Val Asn Leu Met Lys Lys Ile Cys Asp Gln His His Gln Ser  
 290 295 300  
 Arg Ile Ile Thr Val Leu Gly Ser Val His Arg Ser Leu Thr Asp Phe  
 305 310 315 320  
 30 Val Phe Val Ala Gly Asn Trp Ile Ser Lys Phe Phe Ser Ser His Thr  
 325 330 335  
 Arg Gly Ser Leu Asp Pro Tyr Glu Gly Gln Glu Thr Val Val Arg Ala  
 340 345 350  
 35 Met Leu Ala Phe Leu Gln Lys His Leu Asp Leu Lys Glu Asp Tyr Asp  
 355 360 365  
 Gln Trp Asn Asn Phe Ile Glu Gly Ile Gly Pro Ser Leu Thr Pro Gly  
 370 375 380  
 40 Ala Pro His His Leu Ser Ser Leu  
 385 390

## (2) INFORMATION FOR SEQ ID NO: 4:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1665 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: circular  
 45  
 (ii) MOLECULE TYPE: cDNA  
 (vi) ORIGINAL SOURCE:  
 (A) ORGANISM: bovine (Bos taurus)  
 50  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:  
 55

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	GTGACCCAC GCGTCCGAGT TGACCGTCTG GGCTGTTTCT GAGGGTCAAC GTGACTCGCC	60
	GTCAAGTTCA GCCACTGCCC AAGTCGTCGT TCAGTTCAGT TGGTTATGAG ATGGGGGTCA	120
5	ACCAGTCTGT GAGCTTCCCA CCCGTCACGG GACCCACCT CGTAGGCTGT GGGGATGTGA	180
	TGGAGGGTCA GAGCCTCCAG GGCAGCTTCT TTCGACTGTT CTACCCGTGC CAAGAGGCAG	240
	AGGAGACCTC GGAGCAGCCC CTGTGGATTC CCCGCTATGA GTACTGCGCT GGCCTGGCCG	300
10	AATACCTAAA GTTTAATAAG CGCTGGGGGG GGTACTGTT CAACCTGGGT GTGGGATCTT	360
	GTCGCCTGCC TGTTAGCTGG AATGGCCCCT TTAACAACAA GGA CTCTGGA TACCCCTTGA	420
15	TCATCTTCTC TCATGGCATG GGAGCCTTCA GGACAGTGTA TTCAGCCTTC TGCATGGAGC	480
	TGGCTTCTCG TGGCTTTGTG GTTGCTGTAC CAGAGCACAG GGATGGGTCA GCTGCGGCCA	540
	CCTGTTTCTG CAAGCAGACC CCAGAGGAGA ACCAGCCTGA CAATGAGGCC CTGAAGGAGG	600
20	AATGGATCCC CCACCGTCAA ATTGAGGAAG GGGAGAAGGA ATTCTATGTT CGGA ACTACC	660
	AGGTGCATCA GAGGGTGAGC GAGTGTGTGA GGGTGTGAA GATCCTACAA GAGGTCACTG	720
	CTGGGCAGGC CGTTCTCAAC ATCTTGCCTG GCGGATTGGA TCTGATGACC TTGAAGGGCG	780
25	GCATTGACGT GAGCCGTGTG GCTGTAATGG GACATTCATT TGGAGGGGCC ACAGCTATTC	840
	TGGCCTTGGC CAAGGAGATG CAATTTAGGT GTGCTGTGGC TTTGGACGCT TGGATGTTTC	900
30	CTCTGGAGCA TGACTTTTAC CCCACGGCCC GAGGCCCTAT CTTCTTTATC AATGCTGAGA	960
	AGTTCCAGAC AGTGGAGACT GTCAACTTGA TGAAAAAGAT TTGTGACCAG CACCACCAAT	1020
	CCAGGATCAT AACTGTCCTT GGTTCGTTC ATCGGAGTCT AACCGACTTT GTTTTTGTGG	1080
35	CTGGTAACTG GATTAGTAAA TTCTTCTCCA GTCACACCCG TGGAAGCTTG GACCCCTATG	1140
	AAGGTCAGGA GACCGTGGTG CGGGCCATGT TGGCCTTCCT GCAGAAGCAT CTTGACCTGA	1200
	AAGAGGACTA TGACCAGTGG AACAACTTCA TTGAAGGCAT TGGCCCATCA CTGACCCCAG	1260
40	GGGCCCCACA CCATCTGTCC AGCCTGTAGG CACA ACTGGT CATCTTGTGG AAGGTCCCTG	1320
	AGCTGAGTTC CCGTGTGGGG CCTGCCCAGG GATACCCTTG GCCTCCTATC AGGAAGTGAT	1380
45	TGCCATGACC CTTCTGTGTT GATTGAGAGG ATATAATCAC ACTGCTGATT GGTAACGGGG	1440
	TACTTGGATT CTCAGACTTG TCGATCTTAA ACTCATGTTG GGA CTGGGT TCACTTACTG	1500
	ATGGGCAAAC GGCATTCTG AGGACTGAGC CTTAATGGTA TGGAGAACAA ACAGTGGGAT	1560
50	GGGGCTGGGG AAGATCTAAG CCCTAAGCTG GGC ACTATGA GCCCTATAAA CCCAACCAGC	1620
	CAACACCCCTC ACCTTGGGCA AGTATGACTT CTGCAGGTCG ACTCT	1665

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## Claims

1. A protein having activities of a human platelet activating factor acetylhydrolase, and represented by an amino acid sequence represented by the following formula (I) or an amino acid sequence having homology therewith:

Met Gly Val Asn Gln Ser Val Gly Phe Pro Pro Val Thr Gly Pro  
 His Leu Val Gly Cys Gly Asp Val Met Glu Gly Gln Asn Leu Gln  
 Gly Ser Phe Phe Arg Leu Phe Tyr Pro Cys Gln Lys Ala Glu Glu  
 Thr Met Glu Gln Pro Leu Trp Ile Pro Arg Tyr Glu Tyr Cys Thr  
 Gly Leu Ala Glu Tyr Leu Gln Phe Asn Lys Arg Cys Gly Gly Leu  
 Leu Phe Asn Leu Ala Val Gly Ser Cys Arg Leu Pro Val Ser Trp  
 Asn Gly Pro Phe Lys Thr Lys Asp Ser Gly Tyr Pro Leu Ile Ile  
 Phe Ser His Gly Leu Gly Ala Phe Arg Thr Leu Tyr Ser Ala Phe  
 Cys Met Glu Leu Ala Ser Arg Gly Phe Val Val Ala Val Pro Glu  
 His Arg Asp Arg Ser Ala Ala Thr Thr Tyr Phe Cys Lys Gln Ala  
 Pro Glu Glu Asn Gln Pro Thr Asn Glu Ser Leu Gln Glu Glu Trp  
 Ile Pro Phe Arg Arg Val Glu Glu Gly Glu Lys Glu Phe His Val  
 Arg Asn Pro Gln Val His Gln Arg Val Ser Glu Cys Leu Arg Val  
 Leu Lys Ile Leu Gln Glu Val Thr Ala Gly Gln Thr Val Phe Asn  
 Ile Leu Pro Gly Gly Leu Asp Leu Met Thr Leu Lys Gly Asn Ile  
 Asp Met Ser Arg Val Ala Val Met Gly His Ser Phe Gly Gly Ala  
 Thr Ala Ile Leu Ala Leu Ala Lys Glu Thr Gln Phe Arg Cys Ala  
 Val Ala Leu Asp Ala Trp Met Phe Pro Leu Glu Arg Asp Phe Tyr  
 Pro Lys Ala Arg Gly Pro Val Phe Phe Ile Asn Thr Glu Lys Phe  
 Gln Thr Met Glu Ser Val Asn Leu Met Lys Lys Ile Cys Ala Gln

His Glu Gln Ser Arg Ile Ile Thr Val Leu Gly Ser Val His Arg  
 Ser Gln Thr Asp Phe Ala Phe Val Thr Gly Asn Leu Ile Gly Lys  
 Phe Phe Ser Thr Glu Thr Arg Gly Ser Leu Asp Pro Tyr Glu Gly  
 Gln Glu Val Met Val Arg Ala Met Leu Ala Phe Leu Gln Lys His  
 Leu Asp Leu Lys Glu Asp Tyr Asn Gln Trp Asn Asn Leu Ile Glu  
 Gly Ile Gly Pro Ser Leu Thr Pro Gly Ala Pro His His Leu Ser  
 Ser Leu

( I )

2. A DNA encoding said protein of claim 1.
3. An expression vector having said DNA of claim 2.
4. Recombinant host cells transformed by said expression vector of claim 3.
5. A process for the production of a protein having activities of a human platelet activating factor acetylhydrolase, which comprises culturing said recombinant host cells of claim 4 and collecting said protein from the resulting cultured matter.
6. An antibody against said protein of claim 1.
7. A DNA encoding a protein having activities of a bovine platelet activating factor acetylhydrolase, and represented by an amino acid sequence represented by the following formula (III) or an amino acid sequence having homology therewith:

Met Gly Val Asn Gln Ser Val Ser Phe Pro Pro Val Thr Gly Pro  
 5 His Leu Val Gly Cys Gly Asp Val Met Glu Gly Gln Ser Leu Gln  
 Gly Ser Phe Phe Arg Leu Phe Tyr Pro Cys Gln Glu Ala Glu Glu  
 Thr Ser Glu Gln Pro Leu Trp Ile Pro Arg Tyr Glu Tyr Cys Ala  
 10 Gly Leu Ala Glu Tyr Leu Lys Phe Asn Lys Arg Trp Gly Gly Leu  
 Leu Phe Asn Leu Gly Val Gly Ser Cys Arg Leu Pro Val Ser Trp  
 Asn Gly Pro Phe Lys Thr Lys Asp Ser Gly Tyr Pro Leu Ile Ile  
 15 Phe Ser His Gly Met Gly Ala Phe Arg Thr Val Tyr Ser Ala Phe  
 Cys Met Glu Leu Ala Ser Arg Gly Phe Val Val Ala Val Pro Glu  
 His Arg Asp Gly Ser Ala Ala Ala Thr Cys Phe Cys Lys Gln Thr  
 20 Pro Glu Glu Asn Gln Pro Asp Asn Glu Ala Leu Lys Glu Glu Trp  
 Ile Pro His Arg Gln Ile Glu Glu Gly Glu Lys Glu Phe Tyr Val  
 25 Arg Asn Tyr Gln Val His Gln Arg Val Ser Glu Cys Val Arg Val  
 Leu Lys Ile Leu Gln Glu Val Thr Ala Gly Gln Ala Val Leu Asn  
 Ile Leu Pro Gly Gly Leu Asp Leu Met Thr Leu Lys Gly Gly Ile  
 30 Asp Val Ser Arg Val Ala Val Met Gly His Ser Phe Gly Gly Ala  
 Thr Ala Ile Leu Ala Leu Ala Lys Glu Met Gln Phe Arg Cys Ala  
 35 Val Ala Leu Asp Ala Trp Met Phe Pro Leu Glu His Asp Phe Tyr  
 Pro Thr Ala Arg Gly Pro Ile Phe Phe Ile Asn Ala Glu Lys Phe  
 Gln Thr Val Glu Thr Val Asn Leu Met Lys Lys Ile Cys Asp Gln  
 40 His His Gln Ser Arg Ile Ile Thr Val Leu Gly Ser Val His Arg  
 Ser Leu Thr Asp Phe Val Phe Val Ala Gly Asn Trp Ile Ser Lys  
 45 Phe Phe Ser Ser His Thr Arg Gly Ser Leu Asp Pro Tyr Glu Gly  
 Gln Glu Thr Val Val Arg Ala Met Leu Ala Phe Leu Gln Lys His  
 Leu Asp Leu Lys Glu Asp Tyr Asp Gln Trp Asn Asn Phe Ile Glu  
 50 Gly Ile Gly Pro Ser Leu Thr Pro Gly Ala Pro His His Leu Ser  
 Ser Leu

( III )

8. An expression vector having said DNA of claim 7.

9. Recombinant eucaryotic host cells transformed by said expression vector of claim 8.
10. A process for the production of a protein having activities of a bovine platelet activating factor acetylhydrolase, which comprises culturing said recombinant eucaryotic host cells of claim 9 and collecting said protein from the resulting cultured matter.
11. An antibody against a protein having activities of a bovine platelet activating factor acetylhydrolase, and represented by an amino acid sequence represented by the following formula (III) or an amino acid sequence having homology therewith:

```

Met Gly Val Asn Gln Ser Val Ser Phe Pro Pro Val Thr Gly Pro
His Leu Val Gly Cys Gly Asp Val Met Glu Gly Gln Ser Leu Gln
Gly Ser Phe Phe Arg Leu Phe Tyr Pro Cys Gln Glu Ala Glu Glu
Thr Ser Glu Gln Pro Leu Trp Ile Pro Arg Tyr Glu Tyr Cys Ala
Gly Leu Ala Glu Tyr Leu Lys Phe Asn Lys Arg Trp Gly Gly Leu
Leu Phe Asn Leu Gly Val Gly Ser Cys Arg Leu Pro Val Ser Trp
Asn Gly Pro Phe Lys Thr Lys Asp Ser Gly Tyr Pro Leu Ile Ile
Phe Ser His Gly Met Gly Ala Phe Arg Thr Val Tyr Ser Ala Phe
Cys Met Glu Leu Ala Ser Arg Gly Phe Val Val Ala Val Pro Glu
His Arg Asp Gly Ser Ala Ala Ala Thr Cys Phe Cys Lys Gln Thr

```

Pro Glu Glu Asn Gln Pro Asp Asn Glu Ala Leu Lys Glu Glu Trp  
 5 Ile Pro His Arg Gln Ile Glu Glu Gly Glu Lys Glu Phe Tyr Val  
 Arg Asn Tyr Gln Val His Gln Arg Val Ser Glu Cys Val Arg Val  
 Leu Lys Ile Leu Gln Glu Val Thr Ala Gly Gln Ala Val Leu Asn  
 10 Ile Leu Pro Gly Gly Leu Asp Leu Met Thr Leu Lys Gly Gly Ile  
 Asp Val Ser Arg Val Ala Val Met Gly His Ser Phe Gly Gly Ala  
 Thr Ala Ile Leu Ala Leu Ala Lys Glu Met Gln Phe Arg Cys Ala  
 15 Val Ala Leu Asp Ala Trp Met Phe Pro Leu Glu His Asp Phe Tyr  
 Pro Thr Ala Arg Gly Pro Ile Phe Phe Ile Asn Ala Glu Lys Phe  
 20 Gln Thr Val Glu Thr Val Asn Leu Met Lys Lys Ile Cys Asp Gln  
 His His Gln Ser Arg Ile Ile Thr Val Leu Gly Ser Val His Arg  
 Ser Leu Thr Asp Phe Val Phe Val Ala Gly Asn Trp Ile Ser Lys  
 25 Phe Phe Ser Ser His Thr Arg Gly Ser Leu Asp Pro Tyr Glu Gly  
 Gln Glu Thr Val Val Arg Ala Met Leu Ala Phe Leu Gln Lys His  
 30 Leu Asp Leu Lys Glu Asp Tyr Asp Gln Trp Asn Asn Phe Ile Glu  
 Gly Ile Gly Pro Ser Leu Thr Pro Gly Ala Pro His His Leu Ser  
 Ser Leu

(III)